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RESISTANCE TRIALS AGAINST COLLAR ROT OF APPLES CAUSED BY *PHYTOPHTHORA CACTORUM*

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RESISTANCE TRIALS AGAINST COLLAR ROT OF APPLES CAUSED BY *PHYTOPHTHORA CACTORUM*

T. Pl.ziekten 64 (1958): 422–431

RESISTANCE TRIALS AGAINST COLLAR ROT OF APPLES CAUSED BY *PHYTOPHTHORA CACTORUM*[1]

Met een samenvatting: Onderzoek over resistentie tegen stambasisrot van appels, veroorzaakt door Phytophthora cactorum

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INTRODUCTION

In 1953 a hitherto mysterious and in many cases serious trunk rot of 10 to 20 year-old Cox's Orange Pippin trees occurring in orchards in different parts of the Netherlands was found to be caused by *Phytophthora cactorum* (LEB. et COHN) SCHROET. (TEN HOUTEN, 1953). This parasite was already known as the cause of either collar rot or crown rot of apple trees in U.S.A. (BAINES, 1939), Canada (WELSH, 1942), New Zealand (SMITH, 1950), Germany (BRAUN, 1952) and England (SMITH, 1953). The difference between collar rot (the only rot known from Western Europe) and crown rot is that the first develops at the lower parts of the trunk just above the soil surface whereas the second causes a rot of the main roots and of the trunk just below the surface; crown rot is especially known from irrigated orchards. We shall focus our attention on collar rot (fig. 1 and 2), but in citing foreign literature crown rot may also be mentioned, as the disease is the same in principle.

Although preventive measures such as the application of fungicides, good drainage of the orchard, picking up dropped fruits etc. does reduce the number of affected trees, it has not been possible to prevent all spreading of the disease in this way.

Cutting out of the diseased bark and treating the wounds with fungicides has also been successful to some extent. But the most promising means of preventing the establishment of the disease seems to be the use of resistant rootstocks and resistant intermediate scions. That a great difference exists in susceptibility between different rootstocks and different varieties was already known from investigations in the U.S.A. (BAINES, 1939), Canada (FITZPATRICK et al. 1944, MCINTOSH & MELLOR, 1953) and New Zealand (SMITH, 1955).

Breeding for resistance against *Phytophthora cactorum* has been initiated in Canada (MCINTOSH & MELLOR, 1954), but it will be a long time before a resistant desirable variety like Cox's may be obtained.

We concentrated our efforts on the search for resistant varieties. For this purpose a large number of apple varieties were collected in our experimental orchard at Wageningen, and in Limburg some apple orchards with trees about 10 year-old or older were used for comparson in our inoculation trials.

[1] Accepted for publication 4 August 1958.

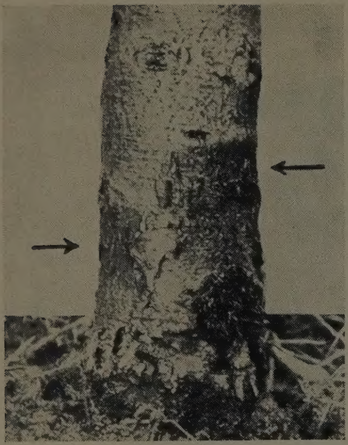

FIG. 1.

Collar rot on Cox's Orange Pippin.
→ diseased area

Stambasis-rot bij Cox's Orange Pippin.
→ aangetast bastgedeelte

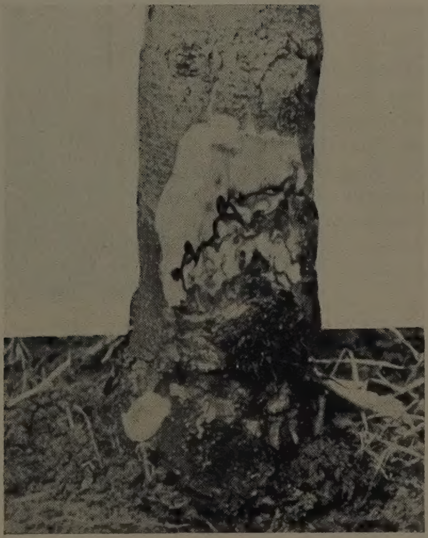

FIG. 2.

Same tree as fig. 1 after cutting away the outer bark tissues.

Dezelfde boom als fig. 1 na wegsnijden van de buitenste bastlagen.

FIG. 3.
Spiral-like inoculation with *P. cactorum* strains on Cox's Orange Pippin tree.
Inoculaties met stammen van P. cactorum, *spiraalsgewijs aangebracht op Cox's.*

Before starting our main inoculation trials the following important questions had to be answered.

1. Which is the most reliable inoculation technique?
2. Is there any difference in virulence between the isolates of *Phytophthora cactorum*?
3. Is there any interaction between different isolates and susceptibility of special varieties, in other words is there any host selectivity among the strains of *Phytophthora cactorum*?
4. What will be the best time of the year for optimal infection?
5. Is there any difference in susceptibility between young and old trees of the same variety?
6. Do individual trees of the same age and variety, growing under the same conditions, vary in susceptibility?
7. Does the rootstock exert any influence on the susceptibility of the scion?
8. Is there any difference in susceptibility between the bark tissues just above the root scion union and those at a higher spot on the main trunk?

1. *The inoculation technique*

In the first trials the method described by BAINES (1939) was used, i.e. a small piece of mycelium and agar was inserted into a deep incision made with the point of a scalpel. The inoculations were covered with cloth and grafting wax. Later a cork borer method was used (BUDDENHAGEN, 1955) making a hole 1 cm in diameter in the bark, down to the wood, inserting a piece of agar with the fungus into the hole, replacing the disc of bark and sealing the wound with vaseline. It appeared to be advisable to fasten the piece of bark with a cotton ribbon in order to prevent displacement (fig. 3). The advantage of the cork borer method is that all wounds have the same size, which allows an easy calculation when measuring the cankers.

2. *Which isolates of the parasite should be used?*

From the literature it was already known that isolates of the parasite differed in pathogenicity. For our resistance trials it was essential that we should use a strain with a high virulence in order to get maximum spread of disease symptoms. On 10 year-old Cox's Orange Pippin trees 20 isolates of *Phytophthora cactorum*, originating from different orchards, were inoculated. There appeared to be a great difference in pathogenicity. An isolate from *Saxifraga cotyledon* from the Central Bureau of Fungus Cultures, Baarn, was almost non-pathogenic, whereas some isolates from Limburg orchards showed a high pathogenicity. For our resistance trials we choose two virulent and one less virulent strain, all isolated from the bark of diseased apple trees.

3. *Is there any host specificity?*

During our experiments it was found that pathogenicity of different isolates was to some extent also dependent on the variety inoculated; i.e. among the strains of *P. cactorum* some host selectivity exists. Analysis of variance of the infection data showed a significant interaction between the isolates and the varieties, but compared with the differences between pathogenicity of the isolates and between the difference in susceptibility between the varieties the effect of this interaction is small.

4. *What is the best season for inoculation?*

In inoculation trials carried out with *P. cactorum* on cut branches of Cox's Orange Pippin, placed in polyethylene bags and incubated at various temperatures at our laboratory, it was found that temperatures above 15 °C and below 30 °C (with an optimum of 20 to 25 °C) were most favourable for canker-size increase (BUDDENHAGEN, 1955). In nature temperature is not the only factor important for infection. Also high humidity is essential for the growth of this fungus. Therefore, in order to establish the best time for inoculation it was necessary to carry out monthly infection trials under orchard conditions. This was done both with *P. cactorum* and with *P. syringae*, a *Phytophthora* species frequently isolated from rotting apple and pear fruits. The results are shown in fig. 4. From the graphs it will be clear that in 1956 June was the best month for ino-

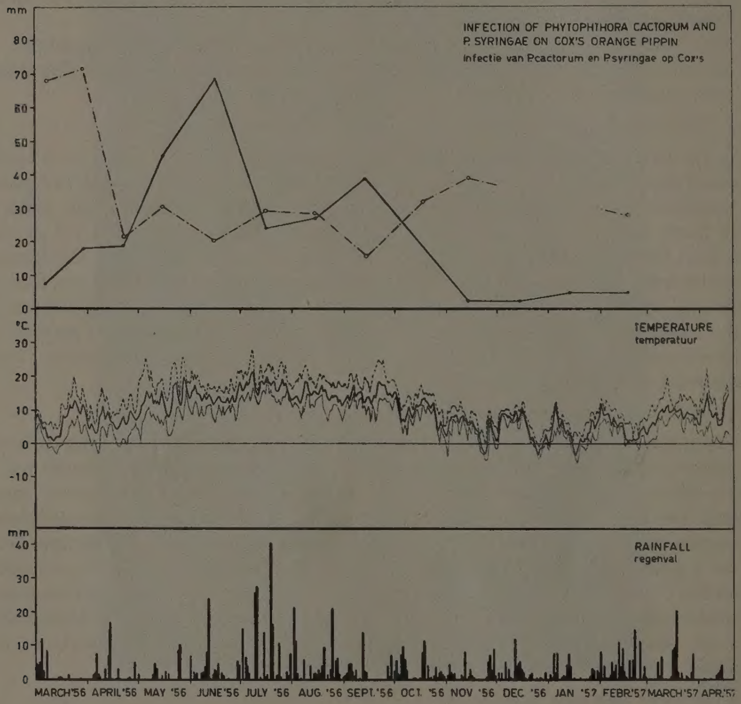

FIG. 4. Seasonal spread of infection of *P. cactorum* and *P. syringae* on Cox's Orange Pippin in relation to temperature and rainfall.

Mate van aantasting van Cox's door P. cactorum *resp.* P. syringae *in de verschillende maanden van het jaar i.v.m. temperatuur en regenval.*

——— in upper graph = spread of infection with *P. cactorum*
mate van aantasting door P. cactorum

-·-·-·- in upper graph = ditto with *P. syringae*
dito door P. syringae

culation with *P. cactorum*, whereas for *P. syringae* March was most suitable. Ir. ROOSJE repeated these experiments in 1957 in the orchard of the Fruit Tree Experiment Station, Zeeland, and again found optimum spread of infection after inoculating *P. cactorum* in June. We shall leave *P. syringae* out of consideration in the rest of this article, because this fungus, although very pathogenic after inoculation, has never been isolated from naturally diseased apple trunks.

5. *Susceptibility of young and old trees of the same variety*

Natural infection of Cox's variety is almost exclusively found in trees 10 year-old or older, but this seems more due to bark conditions favourable for attack by *P. cactorum*, than to a high resistance of the younger tree, although it is true that, after artificial infection, cankers seldom become dangerous in young trees. Contrary to BRAUN & KRÖBER (1958), who only call an infection positive if the canker keeps growing actively until it finally girdles the trunk or the branches, we are of the opinion that every infection is positive where rotting of the bark occurs over some distance. This does not mean, however, that such varieties will also be liable to attack in nature. But it gives us a means of distinguishing between various degrees of susceptibility. This seems of particular interest for the selection of the most resistant intermediate stocks. A comparison of the susceptibility of five commercial varieties growing partly as 17 year-old trees in a Limburg orchard and partly as three year-old trees in our experimental orchard at Wageningen, demonstrated the range of susceptibility to be entirely the same; the order of susceptibility thus seems independent of the age of the trees.

6. *Is there any difference in susceptibility between individual trees of one variety?*

Experiments with several trees of one variety of the same age and growing under the same conditions have shown some variability in resistance; however, generally speaking susceptibility was of the same order within each variety. E.g. 10 trees of the susceptible variety Transparante de Croncels gave the following figures: 39, 42, 47, 38, 42, 46, 44, 45, 44 and 38 (mm length of infection); five trees of Schone van Boskoop had cankers of 24, 22, 21, 26, 23 mm, whereas six Yellow Transparant trees showed 19, 15, 19, 18, 11 and 14 mm of dead bark tissue against 8, 5 and 5 mm for James Grieve, one of the most resistant varieties in our country. All these trees were three year-old and tested in our Wageningen experimental orchard. The same varieties tested in larger numbers of 17 year-old trees in Limburg orchards, although showing more individual fluctuations in susceptibility, still gave the same order of resistance. Therefore it is believed that a rather reliable indication of the amount of resistance may be obtained by inoculating only a few trees of each variety. This seems important for in many cases only two or three trees of one variety are available for these trials.

7. *Does the rootstock exert any influence on the susceptibility of the scion?*

BAINES (1939) finds little or no influence of the rootstock on the resistance of the scion against *P. cactorum*. As no opposite opinion could be found in literature no special attention was paid to the type of rootstock when comparing the resistance of different apple varieties. However, after our experiments were finished we found that scions on seedling rootstocks appeared to be more susceptible than on EM rootstocks (mostly EM IV and EM VII). This was probably due to the more vigorous growth of the trees with seedling rootstocks.

Recent work of NIENHUIS (in BRAUN & KRÖBER, 1958) also suggests influence of the rootstock.

8. *Is the root-scion union more susceptible than other parts of the trunk?*

Infection trials did not show any difference in susceptibility of the bark tissues just above the root-scion union and other places on the trunk or the lower branches. Therefore we have carried out several inoculations in a spiral along the trunk (fig. 3).

RESISTANCE TRIALS

After the various aspects in connection with the reliability of artificial inoculation of apple trees with *Phytophthora cactorum* had been investigated a large number of apple varieties and rootstocks were inoculated.

About 80 apple varieties and eight East Malling (EM) rootstocks have been tested for their resistance to *Phytophthora cactorum* and *Phytophthora syringae*. The latter fungus is frequently isolated from apple or pear fruits, but it has never been isolated from the trunk of a tree. Most varieties were obtained from the Institute for Horticultural Plant Breeding. These trees were for the greater part seven year-old when they were transplanted to our experimental orchard at Wageningen. Other varieties came from Limburg nurseries and were three year-old at the time of inoculation. A series of two year-old intermediate stocks from the Experimental Garden "Boom en Vrucht" at Kesteren was also put at our disposal.

Another series of inoculation experiments was carried out on a number of 17 year-old trees of six commercial varieties in a grower's orchard and on nine year-old EM rootstocks and some other nine year-old commercial varieties in the Experimental Orchard at Beezel (Limburg)[1].

All trees in the main experiment (70 varieties) were inoculated between July 25 and August 3, 1956. The extent of infection was measured between October 29 and November 1, 1956. As we did not know the best infection time before the end of 1956 it later appeared that our inoculations were carried out too late for optimal infection, but the differences found between the varieties seem still sufficient to allow some tentative conclusions. When checking the results the bark was cut away superficially. Then the full length of the discoloured tissue was measured allowing a substraction of 10 mm for the diameter of the bark disc cut out for inoculation.

RESULTS

Preliminary infection trials in 1954 on six varieties in a 17 year-old Limburg orchard with a heavy natural infection of Cox's Orange Pippin trees showed the following order of increasing resistance after artificial inoculation with *P. cactorum* in May: Cox's Orange Pippin, Schone van Boskoop, Jonathan, Yellow Transparant, James Grieve and Manks Codlin. In May 1955 BUDDENHAGEN inoculated 20 trees of each variety and although there were many fluctuations as

[1] The author is much obliged to Ir. S. A. H. M. VAN DE GEIJN and his assistant, Mr. P. J. VAN LIER from the Horticultural Advisory Service Limburg, for their valuable assistance and keen interest in these trials.

an average the same order of increasing resistance was found. Another series of 15 trees each was inoculated in the same orchard in August 1955. Again after four weeks the same sequence of resistance was measured. Also in our main experiment in the experimental orchard at Wageningen using only a few three year-old trees of each variety, we found exactly the same degree of relative resistance, although the trees had been inoculated with a different strain of *P. cactorum*. Manks Codlin was not present in this orchard. Table 1 shows the results for some of the most important varieties tested at Wageningen. James Grieve, Dubbele Zoete Aagt, Dubbele Bellefleur were most resistant. The two Dutch named varieties are now recommended for intermediate stocks. Transparante de Croncels, Sterappel, Red McIntosh, Neerland's Glorie, Reinette van Ekenstein, Sans Pareil and Cox's Orange Pippin all belong to the most susceptible varieties. In a Limburg trial on old trees Mr. VAN LIER found Freiherr von Berlepsch to be highly susceptible (TEN HOUTEN, 1957). This variety and Transparante de Croncels were also among the most susceptible varieties found in Germany (BRAUN & KRÖBER, 1958; SCHMIDLE, 1957). In our 1957 trials in combination with the Horticultural Advisory Service Limburg, Lombartscalville and Ellison's Orange also appeared to be very susceptible. In the 1957 rootstock trials, carried out with Mr. VAN LIER in the Experimental Orchard at Beezel, Limburg, on nine year-old EM rootstocks, EM IX was found highly resistant (which was also reported from N. Zealand and Canada). EM IV and EM VII were also very resistant, but EM XIII and EM XVI were very susceptible, even more susceptible than Cox's Orange Pippin. EM I and EM II were intermediate in susceptibility.

DISCUSSION

When using the cork borer method of inoculation as described by BUDDENHAGEN (1955) it is possible to evaluate the susceptibility of apple varieties for collar rot in a rather reliable way, even if only a few trees of each variety are available. Although several strains of *P. cactorum* exist, having a different virulence and showing some indication of differential pathogenecity, it seems probable that some apple varieties and rootstocks will be resistant towards all physiological strains of the fungus. This opinion is based on the consideration that some varieties and rootstocks have been found resistant or susceptible in infection trials carried out in different parts of the world. It is very improbable that this would be the case without a rather general resistance, as the strains of *P. cactorum* must be different in places at such large geographical distances (Western Europe, U.S.A., New Zealand). In evaluating the results of research workers outside Europe we must take into account that e.g. the Canadian workers test on resistance against crown rot, a disease of the main roots and lower trunk (below soil level), also caused by *P. cactorum*. Crown rot is of particular importance in irrigated orchards. Resistance of varieties and stocks need not be the same to crown rot and collar rot as was clearly demonstrated by SMITH (1955), who found e.g. different reactions below and above ground for the EM rootstocks XII and XIII.

Generally speaking the figures found for resistance in our trials should not be taken too strictly, but the extremes allow us to indicate those varieties which are very susceptible and those which are highly resistant. The latter may receive

consideration as intermediate stocks for such valued susceptible varieties as Cox's Orange Pippin.

TABLE 1. Resistance of some of the more important apple varieties to *Phytophthora cactorum*. Trees inoculated between 25 July and 3 August 1956, results measured between 29 October and 1 November 1956. Wageningen orchard.

Resistentie van enkele van de voornaamste appelrassen t.o.v. Phytophthora cactorum. *Bomen geïnoculeerd tussen 25 juli en 3 augustus 1956, resultaten gemeten tussen 29 oktober en 1 november 1956. Boomgaard te Wageningen.*

Variety *Ras*	Number of trees *Aantal bomen*	Average spread of infection in mm *Gemiddelde aantasting in mm*		
		Isolate I *Schimmel-isolatie I*	Isolate II *Schimmel-isolatie II*	Isolate III *Schimmel-isolatie III*
James Grieve	3	6	5	7
Dubbele Zoete Aagt	3	9	9	8
Wealthy	3	8	9	9
Dubbele Bellefleur	3	10	7	7
Cortland	2	10	6	12
Crimson Gravensteiner	3	12	8	11
Antonovka	2	13	8	8
Zoete Dobbert	2	13	9	9
Virginia Crab	3	12	11	13
Pres. d'Escourt	3	14	11	15
Yellow Transparant	6	18	14	15
Jonathan	7	20	14	16
Schone van Boskoop	5	22	19	15
Wilson's Red June	3	25	9	11
Rambour mortier	2	26	11	20
Pomme d'Or on VII	3	18	10	12
Pomme d'Or on seedling	3	25	12	26
Transp. de Croncels on IV	2	27	13	26
Transp. de Croncels on seedling	3	43	27	42
Neerlands Glorie on IV	3	27	12	23
Neerlands Glorie on seedling	3	38	20	30
Cox's Orange Pippin	3	30	16	23
Reinette Bergamotte	3	29	20	30
Reinette van Ekenstein	1	34	24	35
Sans Pareil	1	26	18	34
Red McIntosh	1	28	25	37
Sterappel	1	48	38	29

SUMMARY

In order to investigate resistance of apple varieties against collar rot (*Phytophthora cactorum*) some information on the following problems had to be gained.

1. Inoculation technique. A cork borer method appeared to be most suitable.
2. Difference in virulence between strains of *P. cactorum* existed, therefore separate inoculations were carried out with three different strains.
3. Host selectivity, although present among the strains of *P. cactorum*, was less than other effects.
4. Monthly inoculation trials indicated June to be the best time for optimal infection.

5. Although some difference was found in susceptibility between young and old trees the variety range of resistance remained the same.
6. Trees of the same variety and age, growing under the same conditions, sometimes differed somewhat in susceptibility but there seems no reason to use large numbers of trees for each variety in collar rot resistance trials.
7. As a rule no influence of the rootstocks on the susceptibility of the scion was found but scions grown on seedling stocks appeared to be more susceptible than those on EM IV and EM VII.
8. No difference in susceptibility was found between the bark tissue just above the root scion union and the bark at a higher spot along the main trunk.

After these points were established 80 apple varieties and 8 EM rootstocks were tested. James Grieve, Wealthy, Dubbele Zoete Aagt and Dubbele Bellefleur belonged to the most resistant varieties whereas Transparante de Croncels, Sterappel, Red McIntosh, Neerland's Glorie, Reinette van Ekenstein, Sans Pareil, Freiherr von Berlepsch and Cox's Orange Pippin were among the most susceptible varieties. EM IX appeared to be the most resistant rootstock. EM IV and EM VII also showed a high resistance. EM XIII and EM XVI were very susceptible, even more susceptible than Cox's Orange Pippin, whereas EM I and II were intermediate in susceptibility. For practical purposes it is recommended to use EM IX, EM IV and EM VII rootstocks. As the very susceptible Cox's Orange Pippin belongs to the most appreciated varieties, it has been advised to use the following resistant intermediate stocks: Dubbele Zoete Aagt and Dubbele Bellefleur. The Cox's Orange Pippin scion should be oculated at about 75 cm above soil level.

SAMENVATTING

Wanneer men de resistentie van appelrassen t.a.v. *Phytophthora cactorum*, de oorzaak van stambasisrot, wil onderzoeken, dient men eerst geïnformeerd te zijn over verschillende daarmee verband houdende problemen, die hier, met de door ons verkregen resultaten, worden opgesomd.

1. De beste methode van inoculatie bleek de door BUDDENHAGEN (1955) ontwikkelde kurkboormethode te zijn.
2. Er bleken verschillen in virulentie tussen de verschillende stammen van *P. cactorum* te bestaan. Daarom werden de te onderzoeken rassen met drie afzonderlijke en in virulentie verschillende isolaties van *P. cactorum* geïnfecteerd.
3. Hoewel er een wiskundig betrouwbare interactie bestaat tussen bepaalde *Phytophthora*-rassen en bepaalde appelrassen, bleek toch het effect hiervan klein t.o.v. de verschillen in aantasting tussen de appelrassen onderling en tussen de rassen van *P. cactorum* onderling.
4. Maandelijks uitgevoerde infectieproeven toonden aan, dat juni de beste maand was om een zo sterk mogelijke uitbreiding van de aangetaste plek te verkrijgen.
5. Bij enkele onderzochte appelrassen, en vooral bij Cox's Orange Pippin, bleek een verschil in vatbaarheid te bestaan tussen jonge en oude bomen, maar dat beïnvloedde de mate van resistentie van de verschillende appelrassen onderling niet.
6. Hoewel er individuele verschillen in vatbaarheid tussen de bomen van één ras

kunnen bestaan, zijn wij toch van mening, dat er bij een nauwkeurig uitgevoerde inoculatie en een ten minste twee maanden durende periode tussen inoculatie en het opmeten van de resultaten, geen reden aanwezig is om grote aantallen bomen van één ras te infecteren ten einde een redelijk betrouwbare uitspraak over de mate van resistentie te kunnen geven. Bovendien zijn meestal om praktische redenen maar weinig bomen voor inoculatie beschikbaar.

7. In de regel werd geen beïnvloeding van de vatbaarheid van de ent door de onderstam waargenomen, behalve bij een serie rassen, die zowel op zaailing als op EM IV, respectievelijk EM VII beschikbaar waren. In dit geval was steeds de vatbaarheid van de rassen groter als ze op zaailing stonden (en dus harder konden groeien).
8. Er werd geen verschil gevonden in vatbaarheid tussen het bastgedeelte vlak boven de entplaats en de hoger langs de hoofdstam gelegen bast. Inoculaties werden dan ook op verschillende plaatsen langs de stam verricht, maar steeds beneden de aanhechting van de gesteltakken.

Nadat wij over bovengenoemde punten enigszins waren ingelicht, werden 80 appelrassen en acht EM onderstammen op hun vatbaarheid getoetst. James Grieve, Dubbele Zoete Aagt en Dubbele Bellefleur behoorden tot de meest resistente rassen, terwijl Transparante de Croncels, Sterappel, Red McIntosh, Neerland's Glorie, Reinette van Ekenstein, Sans Pareil, Freiherr von Berlepsch en Cox's Orange Pippin tot de vatbaarste rassen behoren.

Van de onderzochte EM onderstammen was EM IX het meest resistent, maar ook EM IV en EM VII toonden een hoge mate van resistentie. EM XIII en EM XVI waren zeer vatbaar, zelfs vatbaarder dan Cox's Orange Pippin. EM I en EM II waren tamelijk vatbaar. Gelukkig worden in de praktijk in Nederland in hoofdzaak de drie eerstgenoemde (resistente) onderstammen gebruikt.

Ten einde zeer gewilde, maar voor stambasisrot bijzonder vatbare rassen als Cox's Orange Pippin tegen aantasting te vrijwaren, verdient het aanbeveling van resistente tussenstammen gebruik te maken. Hiervoor zijn in overleg met de N.A.K.B., P.D. en Tuinbouwconsulentschap Limburg voorlopig alleen Dubbele Zoete Aagt en Dubbele Bellefleur aanbevolen. De kwekers van plantmateriaal dienen er op te letten, dat zij de Cox's ent niet te laag boven de grond aanbrengen. Geadviseerd werd hiervoor een hoogte van 70 tot 80 cm aan te houden.

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